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Lyra® Direct Strep ASSAY

For the qualitative detection and differentiation of group A streptococcal and pyogenic group C and G streptococcal nucleic acids isolated from throat swab specimens obtained from patients with signs and symptoms of pharyngitis, such as a sore throat.

For in vitro diagnostic use.

℞ ONLY

A symbols glossary can be found at quidel.com/glossary.

CONTENTS

INTENDED USE

The Lyra Direct Strep Assay is a Real-Time PCR *in vitro* diagnostic test for the qualitative detection and differentiation of Group A β-hemolytic *Streptococcus* (*Streptococcus pyogenes*) and pyogenic Group C and G β-hemolytic *Streptococcus* nucleic acids isolated from throat swab specimens obtained from patients with signs and symptoms of pharyngitis, such as a sore throat. The assay does not differentiate between pyogenic Groups C and G β-hemolytic *Streptococcus*.

All negative test results should be confirmed by bacterial culture because, negative results do not preclude Group A, C or G Strep infection and should not be used as the sole basis for treatment.

The assay is intended for use in hospital, reference, or laboratory settings. The device is not intended for point-of-care use.

SUMMARY AND EXPLANATION

Streptococcal pharyngitis, or "strep throat", is a common bacterial infection found in childhood. Group A streptococci are responsible for the majority of streptococcal pharyngitis cases although other species, such as *S. dysgalatiae*, *S. equi* and *S. canis* may also cause disease.[1] Strep throat affects all age groups but is most common in children between the ages of 5 to 15 years of age.

Streptococci are classified by the production of hemolysis on blood agar and by the use of Lancefield group antigens. Classification by hemolysis can be imprecise due to number of factors (operator experience, incubation conditions of the culture, types of hemolysin produced by the organism, etc.). Lancefield group antigen does not correlate with the species. The molecular taxonomic studies have advanced classification.[2] The beta-hemolytic isolates under Lancefield group A, C, F, and G are subdivided into large and small colony forming groups. The large colony groups possess numerous virulence mechanisms, and are labeled "pyogenic." For specific identification, a serogrouping reagent is used. The large colony Lancefield GCS are variably classified into some of several possible species, namely, *S. dysgalactiae*, *S. equisimilis*, *S. zooepidemicus*, and *S. equi.*[3] The small colony forming groups are classified under Anginosus group that was formerly called "*S. milleri*" group, or *S. intermedius* group. Although these "small colony" organisms may possess the Lancefield group A, C, F, G, and ungroupable antigen, they are commensals and are seldom pathogenic by themselves.

Streptococcal throat infection has an incubation period of 2 to 4 days. Classic symptoms include the abrupt onset of sore throat accompanied by fever, malaise and headache. Physicians diagnose strep throat based on symptoms, physical findings and diagnostic procedures. When strep throat is suspected, prompt and accurate treatment is paramount in order to prevent the occurrence of non-suppurative disease, specifically acute rheumatic fever and post streptococcal

acute glomerulonephritis. Traditional laboratory diagnosis is performed by either rapid antigen testing (RADTs) for Group A streptococcus or culture on sheep blood agar followed by Lancefield group differentiation with latex agglutination. The results of RADTs are often available in less than one hour, while culture results may take 2 to 3 days.

The Lyra Direct Strep Assay allows for the rapid, accurate detection of groups A streptococci and pyogenic group C and G streptococci.

PRINCIPLE OF THE PROCEDURE

The Lyra Direct Strep Assay detects nucleic acids that have been prepared from a patient throat swab processed with a simple heat step. A multiplex real-time PCR reaction is performed under optimized conditions in a single well generating amplicons for group A streptococci or pyogenic group C and G streptococci, and Process Control (PRC). Identification occurs by the use of oligonucleotide primers and probes that are complementary and specific to conserved regions in group A streptococci, pyogenic group C and G streptococci and the PRC. The assay does not differentiate between group C and G streptococci.

Lyra Probe Labels	
Target	**Dye**
Group A streptococci	FAM
Pyogenic group C and G streptococci	CAL Fluor® Red
Process Control (PRC)	Quaser® 670

The following is a summary of the procedure:

1. **Sample Preparation:** Patient throat swab is swirled for approximately 3 to 5 seconds in the Process Buffer to release bacteria from the swab. The Process Buffer is then heated for 10 minutes at 95°C.
2. **Rehydration of Master Mix:** The lyophilized Master Mix is rehydrated using the Rehydration Solution. The Master Mix contains oligonucleotide primers, fluorophore and quencher-labeled probes targeting conserved regions of group A streptococci and streptococci as well as the process control sequence.
3. **Nucleic Acid Amplification and Detection:** 15 µL of the rehydrated Master Mix is added to each plate well. 5 µL of prepared specimen (specimen with PRC) is then added to the plate well. The plate is then placed into the Applied Biosystems® 7500 Fast or 7500 Fast Dx instrument.

   Once the plate is added to the instrument, the Lyra Direct Strep Assay protocol is initiated. This assay is based on Taqman® chemistry, and uses an enzyme with DNA polymerase, and 5'-3' exonuclease activities. During DNA amplification, this enzyme cleaves the probe bound to the complementary DNA sequence, separating the quencher dye from the reporter dye. This step generates an increase in fluorescent signal upon excitation by a light source of the appropriate wavelength. With each cycle, additional dye molecules are separated from their quenchers resulting in an increase in the fluorescent signal. If sufficient fluorescence is achieved, the sample is reported as positive for the detected nucleic acid.

MATERIALS PROVIDED

Cat. #M112
Assay Kit (96 Reactions) – Store at 2°C to 8°C

Component	**Quantity**
Rehydration Solution	1 vial/kit, 1.9 mL
Lyra Direct Streptococci Master Mix	12 vials/kit, 8 reactions/vial
Process Buffer	96 tubes/kit, 300 µL/vial

OPTIONAL MATERIAL

Positive and negative controls for group A and group C+G streptococci (i.e. the laboratory's own internal control materials from isolated and characterized clinical specimen previously submitted for interpretation, which serves as an external processing and extraction control or Quidel Molecular Strep A+G Control Set (M111), which contains positive and negative controls, serves as an external processing and extraction control)

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettors (range between 1 to 10 µL or 2 to 20 µL and 100 to 1000 µL)
- Non-aerosol pipette tips
- 7500 Fast or 7500 Fast Dx
- 7500 Fast or 7500 Fast Dx 96 well PCR plate
- Applied Biosystems optical plate films
- Plate centrifuge for 7500 series 96 well plate
- Dry heating block, capable of heating 1.5 mL tubes at 95°C±1 for 10 minutes

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use
- Performance characteristics of this test have been established with the specimen types listed in the **Intended Use Section** only. The performance of this assay with other specimen types or samples has not been evaluated.
- Using cycling conditions other than those indicated in the Thermocycler Programming Instructions section may give erroneous results.
- Use of this product should be limited to personnel with sufficient training in PCR techniques.
- Treat all specimen/samples as potentially infectious. Follow universal precautions when handling samples, this kit, and its contents.
- Proper sample collection, storage, and transport are essential for correct results.
- Store assay reagents as indicated on their individual labels.
- Wear suitable protective clothing, gloves, eye and face protection when using this kit.
- For accurate results, pipette carefully using only calibrated equipment.
- Thoroughly clean and disinfect all surfaces with a 10% bleach solution followed by molecular grade water.
- Use micropipettes with an aerosol barrier or positive displacement tips for all procedures.
- Avoid microbial and cross contamination of the kit reagents. Follow Good Laboratory Procedures.
- Do not mix reagents from kits with different lot numbers.
- Do not use reagents from other manufacturers with this kit.
- Do not use product after its expiration date.
- Proper workflow planning is essential to minimize contamination risk. Always plan laboratory workflow in a uni-directional manner, beginning with pre-amplification and moving through amplification and detection.
- Use dedicated supplies and equipment in pre-amplification and amplification areas.
- Do not allow cross movement of personnel or equipment between areas.
- Keep amplification supplies separate from pre-amplification supplies at all times.
- Do not open sample tubes or unseal plates post amplification.
- Dispose of amplified material carefully and in accordance with local laws and regulations in order to minimize the risk of amplicon contamination.
- Do not use supplies dedicated for reagent or sample preparation for processing target nucleic acid.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE AND HANDLING OF KIT REAGENTS

- Store the Assay Kit at 2°C to 8°C until the expiration date listed on the outer kit box.
- The rehydrated Master Mix may be kept at room temperature (20°C to 25°C) or at 2°C to 8°C for up to 2 hours, or at –20°C for up to 8 days.
- The rehydrated Master Mix should be recapped, sealed with parafilm, and stored in an upright position. Protect the Master Mix from light during storage.

Indications of Instability or Deterioration of Reagents: Cloudiness of the Rehydration Solution may indicate deterioration of this reagent. Contact Quidel Technical Support for a replacement.

SPECIMEN COLLECTION, STORAGE, AND HANDLING

During clinical studies, the Lyra Direct Strep Assay has been evaluated with Liquid Amies Single Plastic Applicator, Liquid Stuart Single Plastic Applicator, Puritan Liquid Amies Transport System, and Sterile Rayon and Polyester Throat Swabs.

Analytical studies performed with contrived specimens containing group A streptococci, pyogenic group C or G streptococci near LOD (3x LOD) demonstrated that samples can be stored at room temperature or 2°C to 8°C for up to 7 days prior to testing with the Lyra Direct Strep Assay. Specific requirements for shipping specimens should follow recommendations found in section 42 and 49 of the Code of Federal Regulation, CFR and in accordance with applicable national and international transportation regulations.

PROCESSED SPECIMEN STORAGE

Specimens processed in Process Buffer may be stored at 2°C to 8°C, ambient room temperature, –20°C, or –70°C up to 7 days.

INITIAL THERMOCYCLER PROGRAMMING

7500 Fast and 7500 Fast DX Programming Instructions

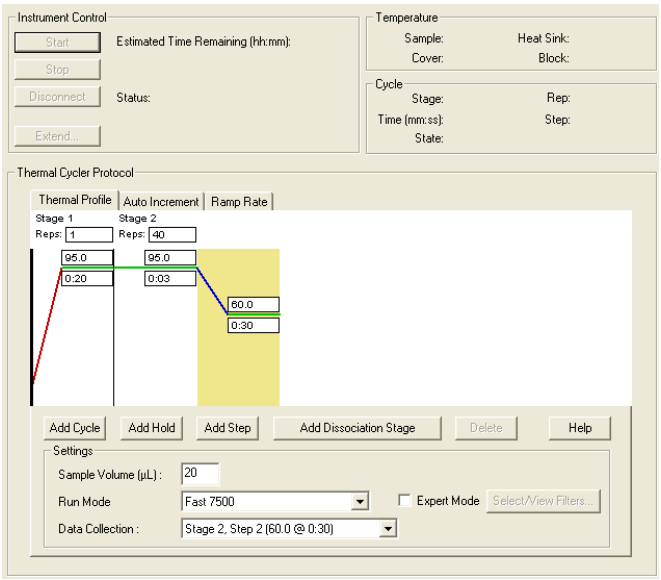
1. Launch the 7500 Fast or 7500 Fast Dx software package.
2. The **Quick Startup document** dialog window will open. Select the **Create New Document** button to start the **New Document Wizard**. Follow each step to initiate the Lyra Direct Strep protocol.
    a. Define Document: Most of the following should be the default setting. If not, change accordingly.
        i. Confirm or enter the following information

Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	Blank Document
Run Mode:	Fast 7500
Operator:	*your operator name*
Comments:	SDS v1.4.1
Plate Name:	Lyra Streptococci

        ii. Select the **Next** button
    b. Select Detectors: New detectors for group A streptococci, group C/G streptococci and the process control (PRC) must be added. For each target, select the **New Detector** button to open the **New Detector** pop-up window. Alternatively, use the **Create Another** button from within the **New Detector** pop-up window for the last two detectors.
        i. Select **(none)** from the **Passive Reference** drop-down menu.
        ii. Enter the following information for each detector.

Name	Reporter Dye	Quencher Dye	Color
Strep A	FAM	(none)	(Select)
Strep C/G	ROX	(none)	(Select)
PRC	CY5	(none)	(Select)

        iii. Select a unique color to represent each detector
        iv. Highlight the new detectors and add to the **Detectors in Document** column using the **Add** button.
        v. Select the **Next** button.
        vi. Select the Finish button without setting any wells.
    c. The wizard will close and the software will open, starting with the **Setup** tab. This will show the sample plate that was set up during the quick start. For the initial set up, nothing needs to be changed here.
    d. Defining the Theromocycler Protocol: Select the **Instrument** tab to set up the Lyra Direct Streptococci PCR cycling times and temperatures. Under **Thermal Profile** there should be a default 2-stage protocol. Each stage will have 3 user-editable text boxes. The top box value represents the number of reps or cycles for that stage. The middle box value represents the temperature (°C) and the lowest box value represents the time (minutes: seconds).

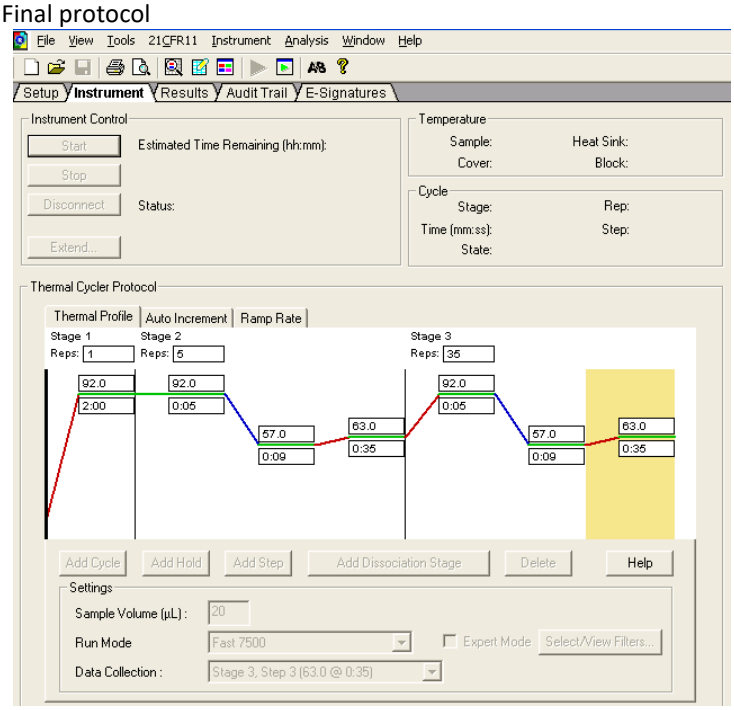


i. Make the following changes to the default **Thermal Cycler Protocol**:
   1. Stage 1
      a. Reps: 1
      b. Temp: 92
      c. Time: 2:00
   2. Stage 2 (3-Step Amplification Stage)
      a. Reps: 5
      b. Step 1
         i. Temp: 92
         ii. Time: 0:05
      c. Step 2
         i. Temp: 57
         ii. Time: 0:09
      d. Step 3
         i. Temp: 63
         ii. Time: 0:35
   3. Select the bar to the right of Stage 2. Select the **Add Cycle** button to add another stage.
   4. Stage 3 (3-Step Amplification Stage)
      a. Reps: 35
      b. Step 1
         i. Temp: 92
         ii. Time: 0:05
      c. Step 2
         i. Temp: 57
         ii. Time: 0:09
      d. Step 3
         i. Temp: 63
         ii. Time: 0:35
   5. If a wrong stage is added the stage can be removed by pressing the **Delete** button after highlighting the stage between the vertical lines

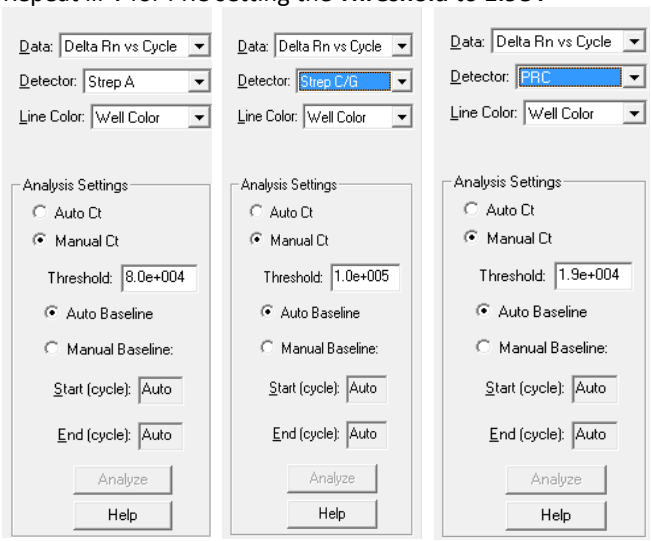
ii. Under **Settings** enter the following:

Sample Volume (µL):	20 (default)
Run Mode:	Fast 7500 (default)
Data Collection:	Stage 3, Step 3(63.0 @ 0:35)
NOTE: Do not check the check box next to 'Expert Mode.'	

iii. Final protocol



e. Set threshold for each analyte
   i. Select the **Results** tab
   ii. Select the **Amplification Plot** tab
   iii. Select Strep A from the Detector tab in the top right corner
   iv. In the **Analysis Settings** block, for group A Strep, set the **Threshold** to **8.0e4**
   v. Select the **Auto Baseline** radio button
   vi. Repeat iii-v for group C/G Strep setting the **Threshold** to **1.0e5**
   vii. Repeat iii-v for PRC setting the **Threshold** to **1.9e4**



f. Save the new protocol as a template for future uses.
   i. At the top of the screen select **File** and then **Save As**
   ii. **Save In:** D:\Applied Biosystems\7500 Fast System\Templates\
   iii. **File name:** 'Lyra Strep'
   iv. **Save as type:** 'SDS Templates (*.sdt)'

g. Exit the software

ASSAY PROCEDURE

Run the following procedures at controlled room temperature of 20°C to 25°C.

Sample Process Procedure

1. Dip the swab into Process Buffer tube and twirl 3 to 5 seconds to remove bacteria from swab and to mix. When ESwab was used for specimen collection, vortex the ESwab collection device for 5 seconds and transfer 50 µL of the ESwab transport medium to a patient-identified Process Buffer tube.
2. Heat the inoculated Process buffer tube in dry heating block for 10 minutes at 95°C±1.

Master Mix Rehydration Procedure

1. Determine the number of specimens to be tested, and obtain the correct number of eight-test lyophilized Master Mix vials for testing.
2. Return unused reagents to the appropriate storage conditions.
3. Open Master Mix carefully to avoid disruption of the pellet.
4. Add 135 µL of Rehydration Solution to the Master Mix.
5. Place vial at room temperature for 1 to 2 minutes to allow rehydration of pellet.
6. Gently pipette up and down 2 to 3 times (avoiding bubble formation) prior to dispensing into the first plate well.
   Note: The rehydrated Master Mix is sufficient for eight reactions.
   Note: The rehydrated Master Mix must be used within 2 hours of rehydration or stored (see Storage and Handling of Kit Reagents above).

PCR Set-up Procedure

1. Add 15 µL of the rehydrated Master Mix to each reaction tube or plate well.
2. Add 5 µL of specimen from the Process Buffer vial into the reaction tubes or plate wells. Mixing of reagents is not required.
   Note: Use a micropipettor with a new non-aerosol tip with each extracted specimen.
3. Close the reaction tubes or seal the plate.
   Note: Quidel suggests each thermocycler run should include a well with External Controls. Run controls in keeping with your lab practices and policies.
4. Centrifuge the reaction tubes or plate for a minimum of 15 seconds. Ensure that all liquid is at the bottom of the plate well or tube.
5. Insert tubes or plate into the thermocycler.

Amplification Protocol on the 7500 Fast and 7500 Fast Dx Thermocycler

1. Switch on 7500 Fast or 7500 Fast Dx.
2. Launch the 7500 Fast or 7500 Fast Dx software package.
3. The **Quick Startup document** dialog window will open.
4. Click on **Create a new document.**
5. Most of the following should be the default setting. If not, change accordingly.

Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	**Lyra Streptococci**
Run Mode:	Fast 7500
Operator:	*your operator name*
Comments:	SDS v1.4.1 (*add more if needed*)
Plate Name:	**YYMMDD-Lyra Streptococci**

6. Set Up Sample Plate
   a. Under the **Setup** and **Plate** tabs the plate setup will appear.
   b. Select all wells that will contain sample, right-click and select the **Well Inspector** from the drop-down menu. When the **Well Inspector** pop-up window opens, select the detectors for Strep A, Strep C/G and PRC.
   c. Use the **Well Inspector** to enter the sample names. Patient IDs may be entered in the Well Inspector window; however it is recommended that this is done prior to resuspending the lyophilized master mix, post run, or using the import function to minimize the time the PCR reactions will sit at room temperature prior to starting the run.
   d. Save the run as **YYMMDD-Lyra Streptococci.sds**.

e. A window will open asking for the "Reason for change of entry." Enter "**Setup**" and any other comments relevant to the run.

7. Starting the PCR
   a. Select the **Instrument** tab.
   b. Insert the 96 well PCR plate into the machine.
   c. Under **Instrument Control**, select the **Start** button to initiate the run.
8. Post PCR
   a. **IMPORTANT:** When the run is finished, press OK. Analyze the data by pressing the **"Analyze"** button in the top menu, and save the file.
   b. Save the file by pressing **Save Document** in the task bar. A window will open asking for the "Reason for change of entry." Enter "**Data analysis post run**" and any other comments relevant to the run.

INTERPRETATION OF RESULTS

Interpretation of Results using the 7500 Fast and 7500 Fast Dx Thermocycler

Interpretation of the Lyra Direct Strep Assay Results on the 7500 Fast and 7500 Fast Dx Thermocycler				
Assay Result	**Detector: Strep A**	**Detector: Strep C/G**	**Detector: Process Control**	**Interpretation of Results**
Negative	Undetermined†	Undetermined†	Ct ≥1, Ct ≤35	No group A streptococcal and no pyogenic group C/G streptococcal DNA detected
Strep A Positive	Ct ≥1, Ct ≤35	Undetermined†	NA*	Group A streptococcal DNA detected
Strep C/G Positive	Undetermined†	Ct ≥1, Ct ≤35	NA*	Pyogenic group C/G streptococcal DNA detected
Strep A and Pyogenic Strep C/G Positive	Ct ≥1, Ct ≤35	Ct ≥1, Ct ≤35	NA*	Group A and pyogenic group C/G streptococcal DNA detected
Invalid	Undetermined†	Undetermined†	Undetermined†	No Strep A or pyogenic Strep C/G and no PRC detected. Retest the same processed sample. If the test is also invalid, obtain a new sample and re-test.
*No Ct value is required for the Process control to make a positive call. †Undetermined = No Ct was reached, therefore the analyte was not detected.				

QUALITY CONTROL

The Lyra Direct Strep Assay incorporates several controls to monitor assay performance.

1. The Process control should be used during sample processing and amplification in the assay. This control is pre-filled in the Process Buffer.
2. Commercially available external positive streptococcal controls may be treated as a patient specimen and should be used in accordance with your lab standards. Previously characterized positive streptococcal specimens may be used lieu of commercial streptococcal controls.
3. A previously characterized negative specimen may be used as an external negative control. This must be treated as a patient specimen and should be performed in accordance with your lab standards.
4. External controls should be used in accordance with the appropriate accrediting organization requirements, as applicable.

LIMITATIONS

- Negative results do not preclude infection with either group A or pyogenic group C and G streptococci and should not be the sole basis of a treatment decision.
- As with other assays of this type, there is a risk of false negative results due to the presence of sequence variants in the amplification targets.

- Improper collection, storage, or transport may lead to false negative results.
- Inhibitors present in the sample and/or errors in following the assay procedure may lead to false negative results.
- Whole blood at concentrations above 0.313% v/v may have a potential for interference.
- For group C and G streptococci, performance has been demonstrated in clinical and analytical studies for *Streptococcus dysgalactiae.* Performance with *Streptococcus equi, Streptococcus canis* has been demonstrated analytically only.
- Negative results for pyogenic beta-hemolytic group C or G streptococci do not preclude the possibility of infection of other beta-hemolytic group C or G streptococci and should be confirmed by culture.
- Additional follow-up testing using the culture method is required if the result is negative and clinical symptoms persist.

EXPECTED VALUES

Performance characteristics of the Lyra Direct Strep Assay using the 7500 Fast Dx was established during a prospective study during the summer and fall of 2013 (August to November 2013). One thousand two hundred ninety three (1293) fresh throat specimens were included in this study at three sites across the United States. A single specimen was collected per patient. Samples were collected on Polyester or Rayon Swab with liquid Amie's or Polyester Swab or Rayon with liquid Stuart's.

The expected value of Group A β-hemolytic Streptococcus (*Streptococcus pyogenes*) and pyogenic Group C and G β-hemolytic Streptococcus (*Streptococcus dysgalactiae, Streptococcus equi, Streptococcus canis*) detected with the Lyra Direct Strep Assay has been calculated for the combined sites based on the age of the patient and overall.

Combined Clinical Study (All Sites) – Expected Values (N=1293)						
Age	Group A β-hemolytic Streptococcus			pyogenic Group C and G β-hemolytic Streptococcus		
	Total #	Total Positive	Prevalence	Total #	Total Positive	Prevalence
≤2 years	37	7	18.9%	37	2	5.4%
3 to 12 years	315	59	18.7%	315	4	1.3%
13 to 21 years	424	32	7.5%	424	61	14.4%
≥22 years	517	34	6.6%	517	21	4.1%
Overall	1293	132	10.2%	1293	88	6.8%

CLINICAL PERFORMANCE

Performance characteristics of the Lyra Direct Strep Assay using the 7500 Fast Dx was established during a prospective study during the summer and fall of 2013 (August to November 2013). One thousand two hundred ninety three (1293) fresh throat specimens were included in this study at three sites across the United States. A single specimen was collected per patient. Samples were collected on Polyester or Rayon Swab with liquid Amie's or Polyester Swab or Rayon with liquid Stuart's. The swabs were inoculated by conventional streak-stab culture technique onto a trypticase soy agar plate containing 5% horse red blood cells. Testing with the Lyra device was performed at the three external laboratories using the same swab that was plated for the culture. All residual specimen transport media from the samples was shipped daily (with cold packs) to a central location. The transport media was cultured using the same testing protocol as that employed by the clinical sites.

The gender and age demographics for each category are listed below.

Combined Clinical Study (All Sites) – Age and Gender Distribution		
Gender	Female	Male
Total	812	481
Age		
≤2 years	14	23
3 to 12 years	173	142
13 to 21 years	269	155
≥22 years	356	161

One thousand two hundred ninety three (1293) fresh throat specimens were cultured for Group A β-hemolytic Streptococcus and tested with the Lyra Direct Strep Assay. The specimens were cultured at the testing sites and the transport media was cultured at a central location. The specimen was considered positive if either the swab or the transport media was positive for β-hemolytic Streptococcus (Composite Culture) and typed as Lancefield group A by latex agglutination. The table below details the overall performance using composite culture results as a reference.

Performance Results of Lyra Direct Strep Assay for Group A β-hemolytic Streptococcus			
Overall Performance (All Sites)			
	Composite Culture		
Lyra Direct Strep Assay	Positive	Negative	Total
Positive	109	24	133
Negative	4	1156	1160
Total	113	1180	1293
			95% CI
Sensitivity	109/113	96.5%	91.3% to 98.1%
Specificity	1156/1180	98.0%	97.0 % to 98.6%
Site 1 Performance			
	Composite Culture		
Lyra Direct Strep Assay	Positive	Negative	Total
Positive	18	1	19
Negative	0	246	246
Total	18	247	265
			95% CI
Sensitivity	18/18	100%	82.4% to 100%
Specificity	246/247	99.6%	97.7 % to 99.9%
Site 2 Performance			
	Composite Culture		
Lyra Direct Strep Assay	Positive	Negative	Total
Positive	54	17	71
Negative	2	556	558
Total	56	573	629
			95% CI
Sensitivity	54/56	96.4%	87.9% to 99.0%
Specificity	556/573	97.0%	95.3 % to 98.1%
Site 3 Performance			
	Composite Culture		
Lyra Direct Strep Assay	Positive	Negative	Total
Positive	37	6	43
Negative	2	354	356
Total	39	360	399
			95% CI
Sensitivity	37/39	94.9%	83.1% to 98.6%
Specificity	354/360	98.3%	96.4 % to 99.2%

One thousand two hundred ninety three (1293) fresh throat specimens were cultured for pyogenic Group C and G β-hemolytic Streptococcus and tested with the Lyra Direct Strep Assay. The specimens were cultured at the testing sites and the transport media was cultured at a central location. The specimen was considered positive if either the swab or the transport media was positive for β-hemolytic Streptococcus (Composite Culture) and typed as Lancefield group C and G by latex agglutination. β-hemolytic isolates that were typed as Group C or G were subcultured and speciated using matrix-assisted laser desorption ionization time-of-flight (MALDI TOF).

The table below details the overall performance using composite culture results as reference.

Overall Performance Results from All Sites of Lyra Direct Strep Assay for Pyogenic Group C and G β-hemolytic Streptococcus			
Overall Performance (All Sites)			
	Composite Culture		
Lyra Direct Strep Assay	Positive	Negative	Total
Positive	67	21	88
Negative	3	1202	1205
Total	70	1223	1293
			95% CI
Sensitivity	67/70	95.7%	88.1% to 98.5%
Specificity	1202/1223	98.3%	97.4 % to 98.9%
Site 1 Performance			
	Composite Culture		
Lyra Direct Strep Assay	Positive	Negative	Total
Positive	29	8	37
Negative	0	228	228
Total	29	236	265
			95% CI
Sensitivity	29/29	100%	88.3% to 100%
Specificity	228/236	96.6%	93.5 % to 98.3%
Site 2 Performance			
	Composite Culture		
Lyra Direct Strep Assay	Positive	Negative	Total
Positive	22	12	34
Negative	2	593	595
Total	24	605	629
			95% CI
Sensitivity	22/24	91.7%	74.2% to 97.7%
Specificity	593/605	98.0%	96.6 % to 98.9%
Site 3 Performance			
	Composite Culture		
Lyra Direct Strep Assay	Positive	Negative	Total
Positive	16	1	17
Negative	1	381	382
Total	17	382	399
			95% CI
Sensitivity	16/17	94.1%	73.0% to 99.0%
Specificity	381/382	99.7%	98.5 % to 100%

ANALYTICAL PERFORMANCE

Limit of Detection (LOD)

The analytical sensitivity (limit of detection or LOD) of the Lyra Direct Strep Assay was determined on using quantified (CFU/mL) stocks of 3 strains of Group A streptococci, 2 strains of pyogenic Group C and 2 strains of pyogenic Group G diluted in a negative matrix. Analytical sensitivity (LOD) is defined as the lowest concentration at which 95% of all replicates tested positive.

Limit of Detection	Bacteria CFU/mL
Group A Streptococcal strain 1 ATCC® 19615 (*Streptococcus pyogenes*)	$6.0x10^2$
Group A Streptococcal strain 2 ATCC 700942 (*Streptococcus pyogenes*)	$2.2x10^3$
Group A Streptococcal strain 3 CCUG 33061 (*Streptococcus pyogenes*)	$1.5x10^3$
Pyogenic Group C Streptococcal strain 1 ATCC 700400 (*Streptococcus equi* subsp. *zooepidemicus)*	$1.7x10^4$
Pyogenic Group C Streptococcal strain 2 CCUG 1483 (*Streptococcus dysgalactiae* subsp. *equisimilis)*	$1.8x10^4$
Pyogenic Group G Streptococcal strain 1 ATCC 12394 (*Streptococcus dysgalactiae* subsp. *equisimilis)*	$1.6x10^4$
Pyogenic Group G Streptococcal strain 2 CCUG 27477	$1.6x10^4$

Analytical Reactivity (Inclusivity)

A study was performed on the 7500 Fast Dx to show that the Lyra Direct Strep Assay detects multiple strains of well-characterized of Group A Streptococci and pyogenic Group C and pyogenic group G Streptococci at equal or less than three times the Limit of Detection (LOD).

The general procedure consisted of testing 11 Group A Streptococcus strains, 10 pyogenic Group C Streptococcus strains and 13 pyogenic Group G Streptococcus strains at equal or less than three times the pre-determined LOD. These strains were a combination of ATCC, CCUG, and Microbiologics characterized strains.

Group A Streptococcal Strains Tested for Inclusivity			
Strains	**Strain ID**	**LOD Level**	**AVG CFU/test**
GAS strain #1	ATCC 19615	0.4x	1.1
GAS strain #2	ATCC 700942	1.4x	3.8
GAS strain #3	ATCC 700952	0.9x	2.6
GAS strain #4	ATCC 12344	1.2x	3.3
GAS strain #5	ATCC 12384	0.7x	1.9
GAS strain #6	ATCC 49399	0.4x	1.2
GAS strain #7	NCIMB 13285	1.8x	5.0
GAS strain #8	CCUG 33061	1.4x	4.0
GAS strain #9	CCUG 33409	0.4x	1.1
GAS strain #10	CCUG 39158	0.5x	1.5
GAS strain #11	CCUG 53553	0.6x	1.6

Pyogenic Group C Streptococcal Strains Tested for Inclusivity			
Strains	**Strain ID**	**LOD Level**	**AVG CFU/test**
Streptococcus dysgalactiae subsp. *equisimilis*	ATCC 12388	1.4x	32.5
Streptococcus equi subsp. *zooepidemicus*	ATCC 700400	1.3x	30.0
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 1483	2.2x	48.8
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 27479	2.1x	48.1
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 27664	1.2x	27.5
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 6713	0.1x	2.1
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 21557	0.7x	16.3
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 27478	2.6x	57.5

Pyogenic Group C Streptococcal Strains Tested for Inclusivity			
Strains	**Strain ID**	**LOD Level**	**AVG CFU/test**
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 27480	1.6x	36.3
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 28238	1.6x	36.3

Pyogenic Group G Streptococcal Strains Tested for Inclusivity			
Strains	**Strain ID**	**LOD Level**	**AVG CFU/test**
Streptococcus dysgalactiae subsp. *equisimilis*	ATCC 12394	0.5x	9.8
Streptococcus canis	ATCC 43497	0.6x	11.5
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 502	0.6x	13.1
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 24070	0.4x	7.5
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 27482	0.3x	6.4
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 27483	2.8x	56.3
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 33645	1.2x	25.0
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 33802	1.0x	19.5
Pyogenic Group G Streptococcal strain (Specific species not identified)	CCUG 1859	N/A	Neat
Pyogenic Group G Streptococcal strain (Specific species not identified)	CCUG 15679	0.3x	7.0
Pyogenic Group G Streptococcal strain (Specific species not identified)	CCUG 15680	1.0x	20.2
Pyogenic Group G Streptococcal strain (Specific species not identified)	CCUG 26147	0.3x	5.3
Pyogenic Group G Streptococcal strain (Specific species not identified)	CCUG 27477	2.3x	46.3

Precision

For the Precision/Within Laboratory Repeatability study, a three (3) member panel consisting of Group A streptococcus, pyogenic Group C streptococcus, and negative sample was tested by two (2) operators, twice a day (2x) for twelve (12) days.

7500 Fast DX Results Summary						
Target		Ct Values and Percent Positive (%)				
		Positive Control	3x LOD	2x LOD	0.3x LOD	Negative Matrix
Group A Streptococcus	Op 1 AVG Ct	20.7	28.0	27.7	32.3	Neg
	Op 2 AVG Ct	20.7	27.7	28.2	32.3	Neg
	Positivity %	100%	100%	100%	45.8%	0.0%
pyogenic Group C Streptococcus	Op 1 AVG Ct	24.9	26.8	27.4	32.6	Neg
	Op 2 AVG Ct	24.3	26.7	27.2	32.6	Neg
	Positivity %	100%	100%	100%	75.0%	0.0%

The Lyra Direct Strep Assay produces results that are highly reproducible on two (2) instruments.

Reproducibility

The reproducibility of the Lyra Direct Strep Assay was evaluated at three (3) laboratory sites (two external, one in-house). Reproducibility was assessed using a panel of four (4) simulated samples that include moderate positive and low positive, high negative and negative Group A streptococcus (*Streptococcus pyogenes*) and pyogenic Group C streptococcus (*Streptococcus equi* subsp. *zooepidemicus*) samples. The panels and controls were processed and tested on the 7500 Fast Dx. Panels and controls were tested at each site by two (2) operators for five (5) non-consecutive days (triplicate testing x

2 operators x 3 replicates x 5 days x 3 sites = 90 results per level for each virus). The LOD values were based on the values obtained in the LOD study.

Reproducibility Results –7500 Fast Dx												
Panel Member ID	Site 1			Site 2			Site 3			Combined Site Data		
	Rate of Detection	AVG Ct	% CV	Rate of Detection	AVG Ct	% CV	Rate of Detection	AVG Ct	% CV	Rate of Detection	AVG Ct	% CV
GAS High Negative	7/30	32.5	1.7	16/30	31.3	4.1	7/30	32.7	4.1	30/90	31.9	4.5
GAS Low Positive	29/30	30.2	4.0	30/30	29.4	4.2	30/30	29.9	8.3	89/90	29.8	4.2
GAS Moderate Positive	30/30	29.2	2.6	30/30	28.5	2.0	30/30	29.0	1.7	90/90	28.9	3.3
GAS Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
GCS High Negative	2/30	34.6	0.7	24/30	32.4	3.4	2/30	32.7	0.6	28/90	32.6	3.6
GCS Low Positive	30/30	31.4	3.1	30/30	28.7	1.4	29/29*	30.4	5.7	89/89*	30.1	5.4
GCS Moderate Positive	30/30	30.3	2.4	30/30	28.0	2.0	30/30	30.1	6.7	90/90	29.5	5.6
GCS Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
GAS Positive Control	30/30	23.4	14.3	30/30	20.2	1.4	30/30	20.6	2.4	90/90	21.3	11.5
GCS Positive Control	30/30	28.1	13.9	30/30	24.0	1.8	30/30	25.2	3.8	90/90	25.8	11.2
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/29*	N/A	N/A	0/89*	N/A	N/A

*One replicate's PRC was not detected. The replicate was reported as invalid.

The data from the combined sites indicates that the Lyra Direct Strep Assay, on the 7500 Fast Dx, generates reproducible results for the detection of Group A Streptococcus (*Streptococcus pyogenes*), Pyogenic Group C Streptococcus (*Streptococcus equi* subsp. *zooepidemicus*), and the internal control.

Analytical Specificity/Cross Reactivity

The analytical specificity with cross-reactive organisms of the Lyra Direct Strep Assay was evaluated in two ways: *in silico* analysis and "wet" testing of actual organisms. The *in silico* analysis involved Basic Local Alignment Search Tool (BLAST) query of the Group A Streptococci and pyogenic Group C/pyogenic group G Streptococci primers against the following sixty (60) organisms:

Non-Cross Reactive microorganisms based on *in silico* Predictions		
Arcanobacterium sp.	Human adenovirus F	*Lactobacillus* sp.[1]
Bacillus sp.	Human adenovirus G	*Legionella pneumophila*
Bacteroides sp.[2]	Human coronavirus 229E	Measles virus
Bordetella sp.	Human coronavirus HKU1	Metapneumovirus
Branhamella sp.	Human coronavirus NL63	*Moraxella* sp.
Burkholderia sp.	Human enterovirus A	Mumps virus
Campylobacter sp.[3]	Human enterovirus B	*Mycoplasma pneumoniae*
Candida sp.	Human enterovirus C	*Neisseria* sp.
Corynebacterium sp.	Human enterovirus D	*Peptostreptococcus* sp.
Cytomegalovirus	Human herpesvirus 1	*Proteus* sp.
Enterobacterio phage MS2	Human herpesvirus 2	*Pseudomonas* sp.
Enterococcus sp.	Human herpesvirus 4	Respiratory syncytial virus Type B
Escherichia coli	Human parainfluenza virus 1	*Saccharomyces cerevisiae*
Fusobacterium sp.	Human parainfluenza virus 2	*Serratia* sp.
Haemophilus sp.	Human parainfluenza virus 3	*Staphylococcus* sp.
Human adenovirus A	Human parainfluenza virus 4	*Treponema* sp.
Human adenovirus B	Influenzavirus A	*Veillonella* sp.
Human adenovirus C	Influenzavirus B	*Yersinia* sp.
Human adenovirus D	Influenzavirus C	*Prevotella oralis*[4]
Human adenovirus E	*Klebsiella* sp.	*Parvimonas micra*[5]

The primers used in the Lyra Direct Strep Assay do not show evidence of cross-reactivity with the above listed microorganisms.

A study was performed on the 7500 Fast Dx to evaluate the performance of the Lyra Direct Strep Assay in the presence of forty-four (44) other microorganisms that might be found in throat specimens. Each potentially interfering microorganism was tested in the presence of 2x LOD Group A streptococcus (2 strains), pyogenic Group C streptococcus strain and pyogenic Group G streptococcus strain Clinically relevant levels of viruses and bacteria are typically 10^6cfu/mL or higher for bacteria and 10^5pfu/mL or higher for viruses.

Non-Cross Reactive microorganisms based on Empirical Testing

Bacteria		
Acinetobacter lwoffii	*Legionella jordanis*	*Streptococcus anginosus*
Arcanobacterium haemolyticum	*Legionella micdadei*	*Streptococcus bovis*
Bacillus cereus	*Legionella pneumophila*	*Streptococcus gordonii* (Virdans type)
Bordetella pertussis	*Moraxella catarrhalis*	*Streptococcus intermedius*
Burkholderia cepacia	*Neisseria gonorrhoeae*	*Streptococcus mitis*
Corynebacterium diphtheria	*Neisseria subflava*	*Streptococcus mutans*
Enterococcus faecalis vanB	*Pseudomonas aeruginosa*	*Streptococcus oralis*
Escherichia coli	*Serratia marcescens*	*Streptococcus pneumoniae*
Fusobacterium necrophorum	*Staphylococcus aureus*	*Streptococcus salivarius*
Haemophilus influenza	*Staphylococcus epidermidis*	*Streptococcus sanguinis*
Klebsiella pneumonia	*Stenotrophomonas maltophilia*	*Streptococcus suis*
Lactococcus lactis	*Streptococcus agalactiae*	*Veillonella parvula*

[1] Includes *L. acidophilus*

[2] Includes *B. ovatus*

[3] Includes *C. rectus*

[4] In NCBI, *Bacteroides oralis* is *Prevotella oralis*.

[5] In NCBI, *Peptostreptococcus micros* is *Parvimonas micra*.

Yeast
Candida albicans

Viruses		
Adenovirus Type 1	Influenza B/Panama/45/90	Rhinovirus Type 15 (1734)
Adenovirus Type 11 (Slobitski)	Influenza C/Taylor/1233/47	
Influenza A/Victoria/3/75/H3N2	Parainfluenza virus 4a	

None of the forty-four (44) other microorganisms that might be found in throat specimens cross-react with the assay.

Microbial Interference

The analytical specificity with interfering organisms of the Lyra Direct Strep Assay was evaluated in two ways: *in silico* analysis and "wet" testing of actual organisms. The same organisms as listed above were evaluated.

The primers used in the Lyra Direct Strep Assay do not show evidence of cross-reactivity with the sixty (60) microorganisms, and therefore are predicted not to cause interference.

None of the forty-four (44) other microorganisms that might be found in throat specimens interfere with the assay.

Interfering Substances

A study was performed on the 7500 Fast Dx to evaluate the performance of the Lyra Direct Strep Assay in the presence of seventeen (17) of potentially interfering/cross-reactive substances, at clinically relevant levels, that might be present in throat specimens.

Interfering Substances Results –7500 Fast Dx						
Substance Name	Substance Final Concentration	Lyra Direct Strep Assay Result				
		Negative Matrix	Group A Streptococcal strain #1	Group A Streptococcal strain #2	Pyogenic Group C Streptococcal strain (*Streptococcus equi* subsp. *zooepidemicus*)	Pyogenic Group G Streptococcal strain (*Streptococcus canis*)
Breath Savers 3 Hour Mint (Spearmint)	10% w/v	Negative	Positive	Positive	Positive	Positive
Cepacol Sore Throat Lozenges	5% w/v	Negative	Positive	Positive	Positive	Positive
Chloraseptic Sore Throat Lozenges	10% w/v	Negative	Positive	Positive	Positive	Positive
Chloraseptic Max Sore Throat Lozenges	1% w/v	Negative	Positive	Positive	Positive	Positive
Children's Dimetapp	15% v/v	Negative	Positive	Positive	Positive	Positive
Crest Pro-Health Clinical Gum Protection	3.13% v/v	Negative	Positive	Positive	Positive	Positive
Halls Cherry Menthol-Lyptus Cough Drops	15% w/v	Negative	Positive	Positive	Positive	Positive
Listerine Ultra-clean Antiseptic Mouthwash	15% v/v	Negative	Positive	Positive	Positive	Positive

Interfering Substances Results –7500 Fast Dx						
Substance Name	Substance Final Concentration	Lyra Direct Strep Assay Result				
		Negative Matrix	Group A Streptococcal strain #1	Group A Streptococcal strain #2	Pyogenic Group C Streptococcal strain (*Streptococcus equi* subsp. *zooepidemicus*)	Pyogenic Group G Streptococcal strain (*Streptococcus canis*)
Listerine Cool Mint Antiseptic Mouthwash	15% v/v	Negative	Positive	Positive	Positive	Positive
Ricola Original Swiss Sugar Free Herb Cough Suppressant Throat Drops	15% w/v	Negative	Positive	Positive	Positive	Positive
Robitussin CF Max	10% v/v	Negative	Positive	Positive	Positive	Positive
Robitussin CF Nighttime	10% v/v	Negative	Positive	Positive	Positive	Positive
Sucrets Complete Lozenges - Cool Citrus	10% w/v	Negative	Positive	Positive	Positive	Positive
Sucrets Complete Lozenges - Vapor Cherry	5% w/v	Negative	Positive	Positive	Positive	Positive
Tic Tac Freshmints	10% w/v	Negative	Positive	Positive	Positive	Positive
Whole Blood*	0.313% v/v	Negative	Positive	Positive	Positive	Positive
Zicam Naturals	15% v/v	Negative	Positive	Positive	Positive	Positive

*Concentrations above 0.313% v/v may cause interference (see limitations above).

None of the seventeen (17) substances interfered with the detection of 2X LOD Group A positive streptococcal or pyogenic Group C and G streptococcal samples. None of the seventeen (17) substances cross-reacted with the Lyra Direct Strep Assay. Whole blood at higher concentrations than noted in the table did demonstrate a potential to cause interference.

Carry-Over and Cross Contamination

Studies were performed the 7500 Fast Dx using a 96-sample panel consisting of 48 high positives and 48 negative specimens. Each high positive specimen contained greater or equal to 1xE06 CFU/ml of a group A and pyogenic group C Streptococcal sample. The negative specimen was negative matrix. The high positive samples were analyzed in series alternating with the negative samples. The testing was repeated over a 5-day period.

Over the course of 5-days, cross-contamination and amplicon carry-over did not occur with the Lyra Direct Strep Assay on the 7500 Fast Dx.

CUSTOMER AND TECHNICAL SUPPORT

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at 740.592.9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com. For services outside the U.S., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

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Revision Changes:

- Add Glossary statement.
- Add Intellectual Property section.

GLOSSARY

REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
Rx ONLY Prescription use only	 Consult e-labeling instructions for use
IVD For *In Vitro* diagnostic use	 Contains sufficient for 96 determinations
CONT Contents/Contains	